INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–1–5–10
http://www.ijab.org

Effect of Variety, Locality and Processing of Coffee Beans on the Detection and Determination of Aflatoxins

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ABSTRACT

Studies were conducted on the detection and determination of aflatoxins after processing in coffee beans originating from various parts of the world (Brazil, Ethiopia, Colombia and Indonesia) using standard AOAC methods. The detection of aflatoxins in green coffee beans could not be successful in overcoming the masked material that covered AFB_2, G_1 and G_2 in all samples under study. However, AFB_1 was the only spot that appeared on the TLC. Due to roasting of coffee beans, the interfering material with aflatoxins increased and even AFB_1 could not be detected by the AOAC methods. An improved procedure which uses silver nitrate to get rid of caffeine and related compounds, proved useful in removing the interfering materials due to which the coffee beans matrix and aflatoxins spots were separated clearly. However, this method failed to remove the interfering materials that masked the area of aflatoxins in roasted coffee beans completely. HPLC was tested as an alternative technique for the detection of aflatoxins. HPLC chromatogram of green coffee beans using the AOAC method showed unidentified intensity peaks and low recovery was obtained. HPLC coupled with the improved procedure of extraction showed that the unidentified peaks due to the matrix of green and decaffeinated coffee beans were eliminated. However, it failed to overcome the problems of the roasted coffee beans especially in the area of AFG_1. An obvious effect of cultivation locality on the matrix of green coffee beans was observed. This effect also appeared in the recovery percentage of aflatoxins. Also, this locality effect did not allow reaching a solid conclusion for the effect of variety.

Key Words: Aflatoxins; Coffee beans; Variety; Origin; HPLC

INTRODUCTION

The exact number of species within the genus coffea (family Rubiaceae), is not known, but is probably around ninety. They are found in the centre of Africa, from the Congo basin to the highlands of Ethiopia. Two species are significant in world trade: coffea arabica and coffea robusta. Arabica coffee developed in highlands of Ethiopia, the climate is cool by tropical standards and rainfall fairly high (Willson, 1999).

Robusta coffee is developed in lowland forest of the Congo River basin extending up to lake Victoria in Uganda. Robusta is less aromatic and higher in caffeine and acidity than Arabica bean. Coffee bean has a complex chemical composition and as variable as nature and man's attendance to its cultivation (Willson, 1999).

Coffee is roasted for about 10 to 20 min at temperatures ranging from 400 to 425ºF. During roasting, the chemical make-up of the coffee beans changes giving much of its aroma and flavor. The caramelize of sugar turns the coffee bean from green to brown (Sievetz & Desrosier, 1979; Massini *et al.,* 1990; Gutierrez *et al.,* 1993; Pittia *et al*., 2001). Large number of volatile compounds in very small amounts give coffee its unique flavour and aroma (Clifford & Willson, 1985; Willson, 1999). To date, over 1000 volatile chemicals have been identified in coffee (Fuster *et al.,* 2000; Clarke & Vitzthum, 2001).

Contamination may occur when either grown crops (Littehoj & Zuber, 1975) or more badly stored harvests are infested by moulds (Jckman, 1985). Among the fungal species, *Aspergillus* and *Penicillium sp.* were found to affect bean color and beverage quality (Batista *et al.,* 2002; Gerrit, 2003). The aflatoxins are potent hepatotoxic and carcinogenic metabolites produced by the fungi *Aspergillus flavus* and *A. parasiticus* (Diener *et al.,* 1987). Betancourt and Frank (1983), found that it is necessary to keep the moisture content (MC) of coffee within a limit of 14.5% to prevent the mold growth during storage.

The published analytical methods for the detection of aflatoxins in green coffee beans using TLC technique are still affected by poor backgrounds and interfering fluorescent material (Levi & Borker, 1968; Levi, 1969; Scott, 1968; Nortowicz *et al.,* 1979; Soliman, 2002, 2004).

The aim of this work was to study the different methods to detect aflatoxins in green coffee beans of different varieties and localities as well as in different coffee products.

MATERIALS AND METHODS

Samples. Indonesian and Brazilian coffee beans which represent the robusta and arabica variety, respectively; Brazilian, Ethiopian and Colombian coffee beans have the same variety (arabica) which represent the different origin

as well as roasted and decaffeinated coffee beans of Colombian were selected.

Preparation of spiked samples. The stock solutions of AFs were mixed with samples of coffee beans under study to get a final concentration of 40 ppb of total aflatoxins (15, 15, 5 and 5 ppb for AFB_1, B_2, G_1 and G_2, respectively).

Sample extraction. The extraction and clean up of aflatoxins were done using the following methods:

A) AOAC for green coffee beans (2000), the finely ground coffee beans (50 g) were mixed with $CHCl_3$ (250 mL) and 10% H_2O and shaken for 30 min, then the extract was filtered through Whatman No. 1 filter paper. A portion of filtrate (50 mL) was loaded onto the florisil column and allowed to flow at a rate 1 drop per second and then the extract was rinsed with tetrahydrofuran (THF). The aflatoxins were eluted with 300 mL acetone-methanol (97:3 v/v) and evaporated to dryness on steam bath under nitrogen.

B) The improved procedure according to Soliman (2004). The sample (50 g of finely ground coffee beans) was shaken with 250 mL $CHCl_3$ and 25 mL $AgNO_3$ solution (25g/100 mL H_2O) for 30 min and filtered through filter paper No 1. The filtrate (75 mL) was mixed with 7.5 mL $AgNO_3$ and shaken for 15 min. The mixture was poured in separating funnel and 50 mL of $CHCl_3$ layer was collected for clean up in Sep-Pak florisil (Soliman, 2004). The THF was used to wash the extract and acetone: methanol (97:3 v/v) was used to elute the aflatoxins and evaporated to dryness on steam bath under nitrogen. The residue was quantitatively transferred to small vial with $CHCl_3$ and evaporated on steam bath under nitrogen and was reserved for TLC and HPLC.

Thin layer chromatography. The thin layer chromatography (TLC) plates (Merck, silica gel G type 60) without fluorescence indicator was used for aflatoxin detection.

Dried residues were dissolved in 200 µL of benzene: acetonitrile (98:2 v/v) and 10 µL of each sample or standard aflatoxins solution were spotted on the same plate. The developing system consists of benzene:alcohol:H2O (46:35:19 v/v/v), two phase solvents system according to Levi (1969) was used, where the bottom layer was used for the saturation of the tank and the upper layer for the developing of the spotted plate. The plate was examined under long wave of the UV light (365 nm).

High performance liquid chromatography (AOAC, 2000). The final extracts from the above were derivativatized by trifluoroacetic acid according to the AOAC (2000). The HPLC used for aflatoxins determination is an Agilent 1100 system equipped with quaternary pump model G1311A, fluorescence detector model G1321A set at 360 nm excitation and 440 nm emission wavelengths and auto-sampler model G 1329A. ODS (Zorbax) column (150 x 4.6 mm) was used for aflatoxins separation. The mobile phase methanol:water:acetonitrile (25:65:10, v/v/v) was isocratically used at flow rate of 1 mL/min. The obtained data were integrated and calculated by Chemstation software program.

Caffeine analysis. Caffeine was extracted and determined in green coffee beans samples according to Madison *et al*. (1976).

RESULTS AND DISCUSSION

The coffee in world trade comes from two major species (arabica & robusta). The effect of interferences compounds on the detection methods of aflatoxins contamination in green coffee beans and its products needs to be focused on. Spiked and aflatoxins-free samples were analyzed by AOAC (2000) method and improved procedure (Soliman, 2004) for green coffee beans.

Effect of Green Coffee Beans Matrix on the Detection of Aflatoxins

Effect of green coffee beans verities. To study the effect of variety matrix on the detection of aflatoxins, samples of Indonesian (robusta variety) and Brazilian coffee arabica variety coffee beans were spiked with aflatoxins at a level of 40 ppb and analyzed with above mentioned procedures.

Both the arabica (Brazilian) and robusta (Indonesian) varieties, when extracted with the AOAC method showed that AFB_1 was the only spot that appear in the TLC plate (Fig. 1). In Indonesian coffee beans an extraneous fluorescent spot appeared below the R_f of AFG_2. However, this fluorescent spot did not appear in the extract of Brazilian coffee beans.

This fluorescent spot appearing in both spiked and aflatoxins-free coffee beans of the robusta variety may indicate the relation of variety to each spot. Soliman (2004) showed that the fluorescent spot is a compound which may be related to the matrix of coffee beans and is most probably a caffeine compound. This result matches with the high level of caffeine in robusta (16.3 mg/g) compared with arabica (6.9 mg/g) (Table I). In this regard Willson (1999) reported that the caffeine and acidity content of the robusta coffee beans are about twice that of arabica.

An improved procedure reported by Soliman (2004), which uses silver nitrate as the aqueous solution to overcome the different problems associated with the AOAC procedure, was successful to remove the interfering materials in green coffee beans. So a good background was obtained on TLC and the four types of aflatoxins were separated clearly (Fig. 2). This result is in agreement with Scott (1969) and Soliman (2004) who reported that silver nitrate can be used to get rid of the caffeine and related compounds.

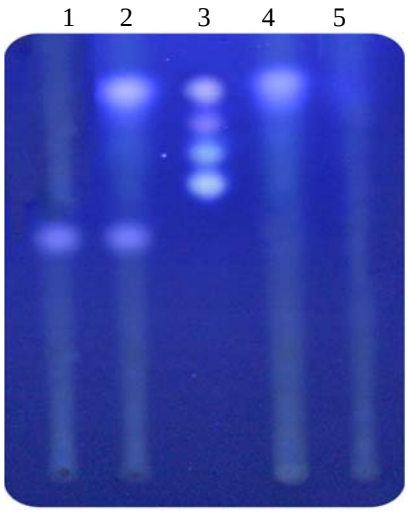
Table I. The level of caffeine (mg/g) in four types of green coffee beans

Item	Brazilian	Colombian	Ethiopian	Indonesian
Caffeine (mg/g)	6.9±0.89	5.4±0.92	8.2±1.80	16.3±2.60

Table II. Comparison of aflatoxins percent recoveries of green; roasted and decaffeinated coffee beans using the improved procedure

Matrix	B_1	B_2	G_1	G_2	Total
Robusta Indonesian-green	70.0±4.2	81.5±5.8	67.0±8.3	80.0±4.5	74.6±4.7
Arabica Brazilian-green	89.0±4.2	86.7±6.2	87.8±5.8	91.4±4.5	88.7±5.2
Ethiopian-green	88.5±7.2	86.5±7.9	62.9±7.5	80.8±6.7	79.7±5.3
Colombian-green	62.3±9.2	78.2±6.1	40.2±7.4	71.7±8.1	63.1±6.7
Colombian-decaffeinated	81.5±7.9	85.6±8.4	46.4±8.5	75.7±6.8	72.3±7.1
Colombian-roasted	63.9±7.3	78.9±8.7	42.8±5.5	71.4±8.7	64.3±7.6

Fig. 1. Effect of green coffee beans variety on detection of aflatoxins using the AOAC method. 1, Aflatoxins-free Indonesian coffee beans; 2, Spiked-Indonesian coffee beans with aflatoxins; 3, Aflatoxin standards; 4, Spiked-Brazilian coffee beans with aflatoxins; 5, Aflatoxins-free Brazilian coffee beans



Effect of green coffee beans origin. To study the effect of coffee beans origin (locality of cultivation) on the detection of aflatoxins, samples of the same variety (arabica) were selected to represent different origin of cultivation. These samples were Brazilian, Ethiopian and Colombian coffee beans.

Fig. 3 shows that the AOAC procedure could not overcome the masked material that covered the area of AFB_2, G_1 and G_2 in all samples under study and AFB_1 was the only spot which appeared in these samples. Slight fluorescent spot at R_f below AFG_2 appeared in Ethiopian coffee beans which means that higher content of caffeine may be present in Ethiopian samples. This finding is confirmed by the higher level of caffeine in Ethiopian samples that was detected by HPLC in the current study (8.2 mg/g) compared with those of Brazilian and Colombian (6.9 and 5.4 mg/g, respectively) (Table I).

Fig. 2. Effect of green coffee beans variety on detection of aflatoxins using an improved procedure. 1, Aflatoxins-free Indonesian coffee beans; 2, Spiked-Indonesian coffee beans with aflatoxins; 3, Aflatoxin standards; 4, Spiked-Brazilian coffee beans with aflatoxins. 5, Aflatoxins-free Brazilian coffee beans.

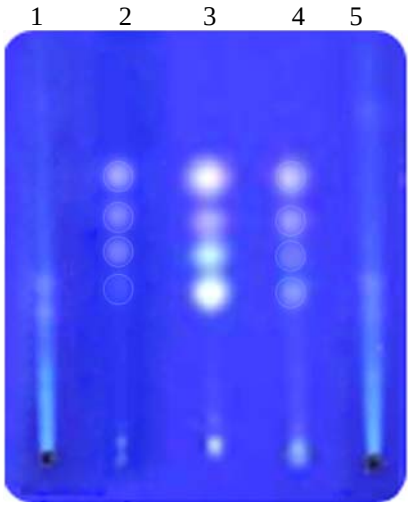
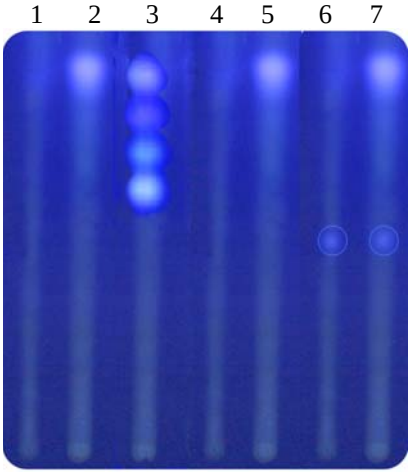


Fig. 3. Effect of origin green coffee beans on detection of aflatoxins using the AOAC method. 1, Aflatoxins-free-Brazilian coffee beans; 2, Spiked Brazilian coffee beans with aflatoxins; 3, Aflatoxins standard; 4, Aflatoxins-free-Colombian coffee beans; 5, Spiked Colombian coffee beans with aflatoxins; 6, Aflatoxins-free-Ethiopian coffee beans; 7, Spiked Ethiopian coffee beans with aflatoxins.



Using the improved procedure of Soliman (2004), the masking material was not observed in the same samples (Fig. 4). Four spots of aflatoxins were detected clearly in Brazilian coffee; however, AFG_1 did not appear in Colombian and Ethiopian coffee beans on TLC.

The percentage recoveries of aflatoxins in Indonesian, Brazilian, Ethiopian and Colombian green coffee beans are

Fig. 4. Effect of origin green coffee beans on detection of aflatoxins using an improved procedure. 1, Aflatoxins-free-Brazilian coffee beans. 2, Spiked Brazilian coffee beans with aflatoxins; 3, Aflatoxins standard; 4, Aflatoxins-free-Colombian coffee beans; 5, Spiked Colombian coffee beans with aflatoxins; 6, Aflatoxins-free-Ethiopian coffee beans; 7, Spiked Ethiopian coffee beans with aflatoxins.

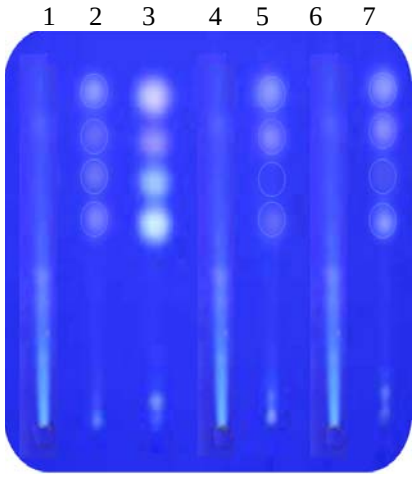


Fig. 5. Effect of locality and variety variation on percentage recovery of total aflatoxins.

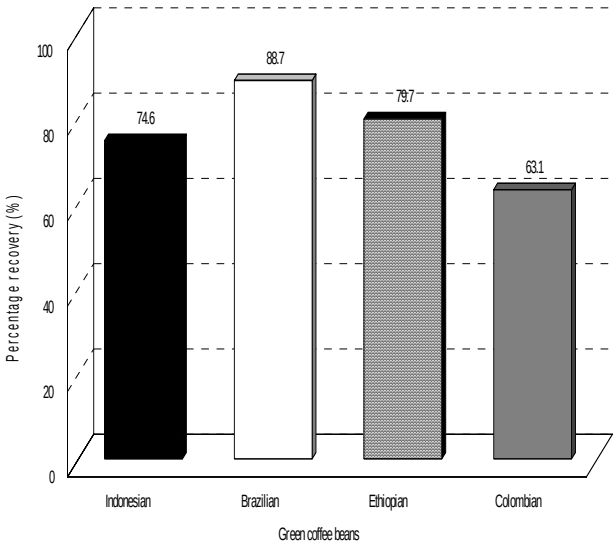


Fig. 6. Effect of processing coffee beans on detection of aflatoxins using the AOAC method. 1, Aflatoxins-free Colombian roasted coffee beans; 2, Spiked-Colombian roasted coffee beans with aflatoxins; 3, Aflatoxins standard; 4, spiked-Colombian decaffeinated coffee beans with aflatoxins; 5, Aflatoxins-free Colombian decaffeinated coffee beans.

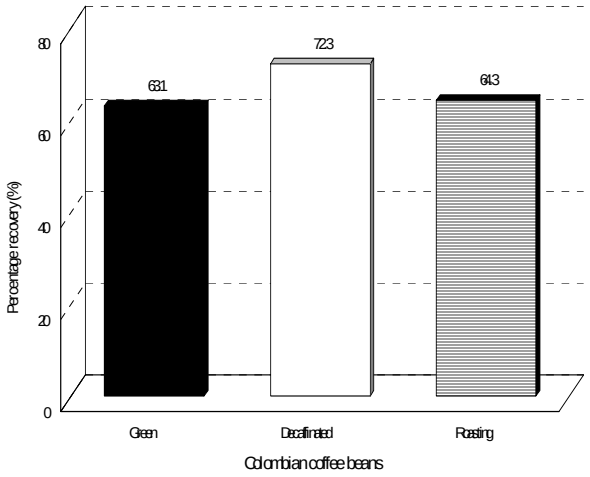
Fig. 7. Effect of processing coffee beans on detection of aflatoxins using an improved procedure. 1, Aflatoxins-free Colombian roasted coffee beans; 2, Spiked-Colombian roasted coffee beans with aflatoxins; 3, Aflatoxins standard; 4, Spiked-Colombian decaffeinated coffee beans with aflatoxins; 5, Aflatoxins-free Colombian decaffeinated coffee beans.

presented in Table II and Fig. 5. No general conclusion of the variety effect could be inferred due to an apparent effect of the locality of cultivation, which may mask such varietal effect. So the higher recovery of Brazilian coffee beans (88.7%) compared to Indonesian coffee beans (74.6%) may not be due to the effect of variety variation rather than the effect of locality which is obviously observed in comparing the same variety, the Brazilian coffee beans (88.7%) and the Colombian samples (63.1%).

Effect of coffee processing on the detection of aflatoxins. Processing (roasting and decaffeination) of coffee beans causes the development of compounds with new flavor and color that may interfere with the detection of aflatoxins in the samples of processed coffee beans (Lerici & Nicoli, 1990; Pitta *et al.*, 2001).

Roasting of coffee beans. To study the effect of roasting on the efficiency of aflatoxins detection procedure, roasted Colombian samples were used. As expected, the AOAC procedure failed to separate the aflatoxins spots from the

Fig. 8. Effect of processing on percentage recovery of total aflatoxins



masked material that covered the area of aflatoxins spots (Fig. 6). The interfering materials that masked the area of aflatoxins were less intense when the improved procedure was used to extract the aflatoxins in roasted Colombian coffee beans. The spots of AFB_1 and B_2 although appeared but not clearly (Fig. 7).

The results obtained in the current study confirmed that the AOAC method (2000) is described only for green coffee beans and this method is not suitable for the detection of aflatoxins in roasted coffee beans.

The results further showed that although the improved procedure succeeded to resolve the problems of the detection of aflatoxins in green coffee beans, it failed to overcome the problems of pigmented compounds produced during roasting process. In this regard, Sievetz and Desrosier (1979) stated that roasting coffee beans causes marked chemical, physical, structural and sensorial changes. Therefore, the need to establish a specific method for the detection of AFs in roasted coffee beans is highly recommended.

Decaffeination of coffee beans. Colombian-decaffeinated coffee beans were used to study the effect of decaffeination on the detection of aflatoxins using the above mentioned procedures (AOAC and the improved one). Fig. 6 showed poor background on TLC plate with AOAC extraction, however better background was observed when the improved procedure was used (Fig. 7).

Data in Table II and Fig. 8 showed that the best recovery was obtained from the decaffeinated samples (72.3%) compared with the green (63.1%) and roasted coffee beans (64.3%). The AFB_2 recorded the highest recovery (85.6, 78.9 & 78.2%) for decaffeinated, roasted and green coffee beans, respectively. On the other hand the lowest recovery was recorded for AFG_1 (46.4, 42.8 and 40.2, for decaffeinated, roasted and green coffee beans, respectively) compared with the other aflatoxins.

Fig. 9. HPLC chromatogram of aflatoxins spiked green coffee bean sample extracted by AOAC method for green coffee bean.

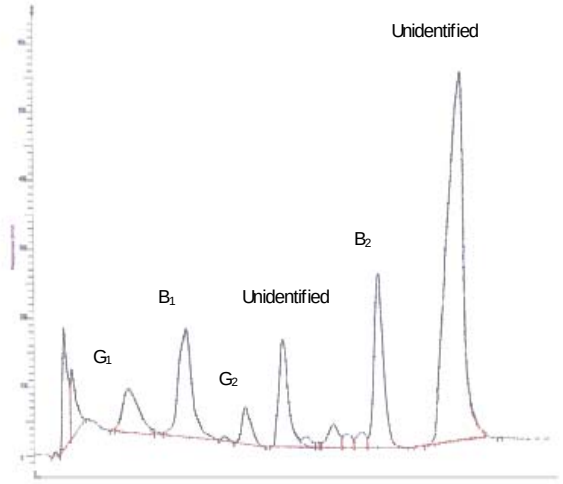
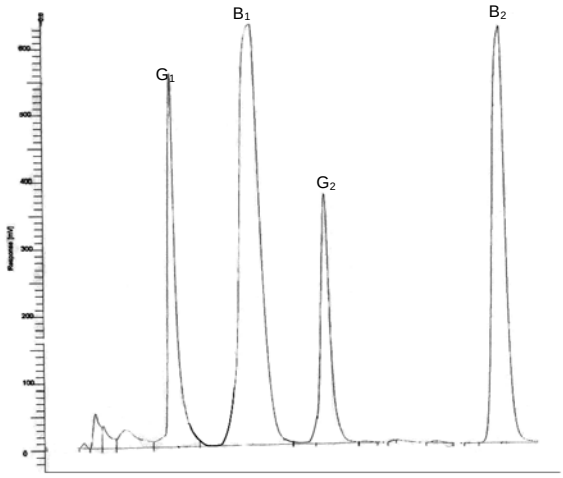


Fig. 10. HPLC spiked green coffee beans chromatogram of aflatoxins extracted by improved procedure.



HPLC as an alternative technique for the detection of aflatoxins. HPLC was tested as an alternative technique to avoid problem of the interfering compounds on TLC. The HPLC chromatogram of green coffee beans using the AOAC method (Fig. 9) showed two unidentified high intensity peaks (Soliman, 2004). These peaks were well resolved from the aflatoxins peaks. This showed that HPLC determination coupled with AOAC as a method of extraction could resolve the problem of TLC; however, the low recovery of the AOAC method (46.1%) (Soliman, 2004) may prevent such approach. When the improved procedure was used for the extraction of green and decaffeinated coffee beans, the unidentified peaks due to the matrix of the same above mentioned samples was

Fig. 11. HPLC chromatogram of aflatoxins spiked decaffeinated coffee beans extracted by the improved procedure

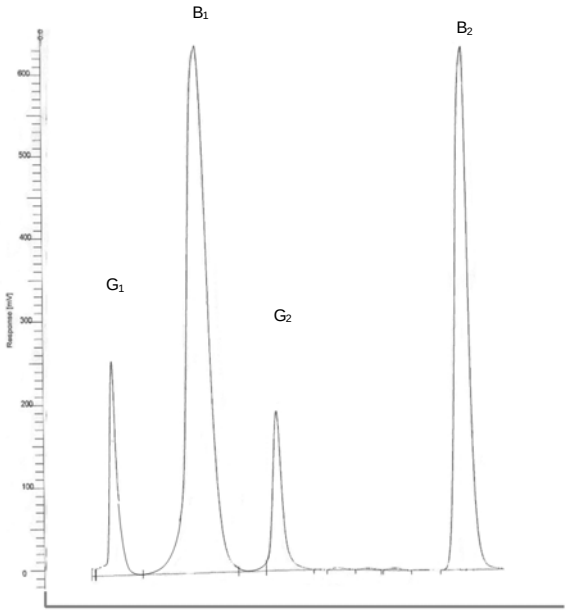
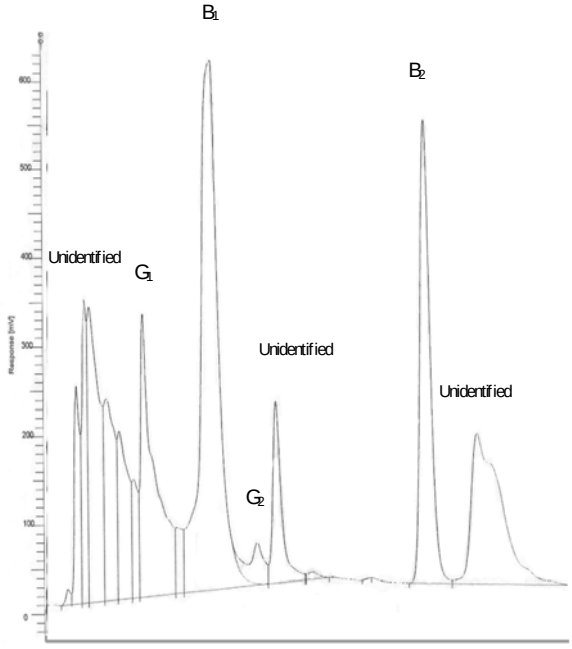


Fig. 12. HPLC chromatogram of aflatoxins spiked roasted coffee beans extracted by the improved procedure



eliminated (Fig. 10 & 11). The HPLC chromatogram of aflatoxins in roasted coffee bean samples showed a lot of interference peaks were appeared especially in the area of AFG_1 (Fig. 12). This result show that although the improved procedure coupled with HPLC resolve the problems of the TLC technique during the detection of aflatoxins in green and decaffeinated coffee beans, however, the problem of roasted coffee beans still exited (Fig. 12). This still needs a special method for roasted coffee beans.

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(Received 01 November 2004; Accepted 14 December 2004)